Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 3416–3421, April 1996
Microbiology

Hyphal development in *Neurospora crassa*: Involvement of a two-component histidine kinase

(filamentous fungi/signal transduction/osmoregulation)

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Contributed by Melvin I. Simon, December 11, 1995

ABSTRACT **Two-component signal transduction systems are most often found in prokaryotic organisms where they are responsible for mediating the cellular responses to many environmental stimuli. These systems are composed of an autophosphorylating histidine kinase and a response regulator. We have found evidence for the existence of two-component histidine kinases in the eukaryotic filamentous fungus *Neurospora crassa* based on screening with degenerate primers to conserved regions of these signaling proteins. Subsequent cloning and sequencing of one member of this newly discovered group, *nik-1*$^+$, shows that the predicted protein sequence shares homology with both the kinase and response regulator modules of two-component signaling proteins. In addition, the N-terminal region of the protein has a novel repeating 90-amino acid motif. Deletion of the *nik-1*$^+$ gene in *N. crassa* results in an organism that displays aberrant hyphal structure, which is enhanced under conditions of high osmostress. Increased osmotic pressure during growth on solid medium leads to restricted colonial growth, loss of aerial hyphae formation, and no subsequent conidiophore development. This finding may have implications for mechanisms of fungal colonization and pathogenicity.**

The information carried by signal transduction cascades is often transmitted through reversible protein phosphorylation involving modification of serine, threonine, and tyrosine residues of signaling proteins in eukaryotic cells. It has become apparent that these proteins are often comprised of a number of basic conserved modules such as SH2 and SH3 domains placed together in a fashion that functions in the particular signaling circuit at hand (1). Alternatively, prokaryotic organisms have utilized phosphorylation of histidine and aspartic acid residues in proteins to mediate the information flow of their signaling circuits. This is typified by the name two-component signal transduction system, where the first component is an autophosphorylating histidine kinase whose activity is modulated in response to a specific stimulus and the second component is called a response regulator. The response regulator serves as a substrate for the histidine kinase and becomes phosphorylated on an aspartate residue after phosphoryl transfer from the histidine residue of the kinase. Phosphorylation of the response regulator controls its function, which could be enzymatic activity, DNA binding, or protein–protein interaction (1–3).

Two-component systems can most readily be identified through amino acid sequence homologies. The kinase domain is a module of ≈250 amino acids that has 5 conserved blocks of amino acid sequence (see refs. 1–3). The H box is the site of histidine autophosphorylation; the F, G1, and G2 boxes are thought to be involved in nucleotide binding; and the function of the N box remains unknown (1–3). Similarly, the response regulator domain can be identified from the number and spacing of conserved aspartate, lysine, and hydrophobic residues in a module of ≈120 amino acids. These two signaling modules can be found in a variety of contexts within more complex signaling proteins, mirroring the modularity of eukaryotic signaling proteins. There are numerous processes regulated by two-component systems including bacterial chemotaxis and many gene regulation events in response to stimuli such as changing osmolarity, oxygen, nitrogen, and phosphorous levels (1–3).

The notion that two-component systems are found only in bacteria has been shown to be incorrect. Recently, ETR1 and ERS, proteins involved in ethylene signal transduction in *Arabidopsis thaliana*, have been shown to be members of the histidine kinase family (4, 5). Also, two signal transduction proteins involved in regulating the HOG1 MAPK cascade in *Saccharomyces cerevisiae* have been identified as a two-component histidine kinase SLN1 and its response regulator SSK1 (6, 7). *S. cerevisiae* also contains a second response regulator, SKN7, that may be involved in cell wall assembly, although in this case the cognate histidine kinase is unknown (8). We have wondered whether this class of proteins could be found in other eukaryotes as well. By aligning members of the histidine kinase family, we could design oligonucleotide primers to amplify the kinase domain. Two new members of the histidine kinase family have been found in the filamentous fungus *N. crassa*. We have cloned one member of this family, *nik-1*$^+$, and we show that it plays a role in hyphal development in *N. crassa*.

MATERIALS AND METHODS

Strains. The *N. crassa* strain 74-OR23-1VA (mating type A, 74A) was used as the source for purification of genomic DNA and mRNA unless otherwise noted and was obtained from the Fungal Genetics Stock Center (Kansas City, KS, no. 2489). Genomic DNA for PCR was prepared from isolated nuclei and was a generous gift of G. Turner (University of California, Los Angeles). The *N. crassa* strain used for electroporation is called Stadler (mating type **a**) and is *pdx-1his-2Δmtr* and was obtained from D. Stadler (University of Washington). Handling techniques and growth media for *N. crassa* are described by Davis and deSerres (9).

Molecular Biological Manipulations. All common molecular biological manipulations were carried out according to standard methods (10). Reverse transcriptase coupled (RT)-PCR was performed as described except that the primer concentrations were increased to 1.5 μM (11). Sequencing of DNA templates was done with Sequenase (United States

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Abbreviations: RT-PCR, reverse transcriptase coupled PCR; pdx, pyridoxine hydrochloride.

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§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U50264 (genomic sequence) and U50263 (cDNA sequence)].

Biochemical) or by *Taq* cycle sequencing using dye primer or dye terminator chemistry on an automated sequencer (Applied Biosystems model 373) according to the manufacturer's specifications.

PCR Amplification of *N. crassa*. Alignment of histidine kinase members was accomplished using the PILEUP program from the Wisconsin Genetics Computer Group (GCG) package. The following primer to the H box (H1A) was made [CA(T/C)GAI(A/T/C)TI(C/A)GIACICC] and served as the forward PCR primer. The reverse primers were synthesized to code for the N box: N1A, GT(A/G)AA(T/C)TTIAIIGC(A/G)TT; N2A, GC(A/G)TTIC(T/C)IACIA(G/A)(G/A)TT (I is deoxyinosine). All primers were purified by electrophoresis through an acrylamide gel (20%/7 M urea) followed by purification over a Sep-Pak C_{18} column. PCR mixtures contained 2.5 μM each primer; 1.4 μg of genomic DNA; 2.5 mM each dATP, dGTP, dCTP, and dTTP in 10 mM Tris·HCl pH 8.3/50 mM KCl/0.001% gelatin/0.5 unit of Amplitaq (Perkin–Elmer/Cetus) in a total vol of 100 μl. Primers, buffer, and DNA templates were mixed and heated (95°C for 10 min) in a Perkin–Elmer/Cetus thermocycler and then cooled to 4°C. Nucleotides and polymerase were added and reactions mixtures were cycled 25–30 times at 94°C (1 min), 40°C (1 min), and 72°C (1 min) followed by a 10-min extension at 70°C. Amplification products were purified by electrophoresis through a 1.5% agarose gel, cloned into a T-vector (Promega), and subsequently sequenced. Sequence analysis was done using Sequencher (version 2.1, Gene Codes, Ann Arbor, MI) and the GCG package. Homology searches and sequence comparisons were performed using the BLASTX and BESTFIT programs of the GCG package.

Genomic Cloning of *nik-1*$^+$. Approximately 150,000 clones from a λJ1 genomic library (constructed by M. Orbach, obtained from the Fungal Genetics Stock Center) were screened with a randomly primed *nik-1*$^+$ PCR product (Prime-a-gene, Promega) obtained by amplification of *N. crassa* genomic DNA with the H1A and N2A primers. Positive clones were isolated, digested with *Bam*HI and grouped by common hybridization patterns. A 5.5-kb *Bam*HI fragment was cloned into *Bam*HI-digested pUC18 to yield pHK1. This clone contained the kinase domain as verified by sequence analysis. pHK1 served as a template for the genomic sequencing of *nik-1*$^+$, which was accomplished by a combination of primer walking, sequencing small subclones, and sequencing deletion subclones in M13 (12).

cDNA Cloning of *nik-1*$^+$. Approximately 10^6 clones from the Orbach and Sachs cDNA library (13) (obtained through FGSC) were screened with the *nik-1*$^+$ kinase domain PCR product. Two sets of clones were obtained, M1 and M10, which started at nucleotides 3285 and 3760 of the genomic sequence, respectively. These clones were sequenced entirely on both strands. RT-PCR was used to walk upstream of the 5′ end of M1 and subsequently clone the entire cDNA coding for *nik-1*$^+$. All RT-PCR products were cloned into a T-vector and sequenced. Primer extension with the oligonucleotide NK45 (GAGAGCTGGCTGATCTGTTG) revealed the transcription start site 969 bases upstream of the initiator AUG of the Nik-1 protein.

RNA Preparation. Total RNA was prepared from *N. crassa* at various stages of development as described (14). For mycelial RNA, cultures were harvested after 8–16 h of growth in Vogel's 1× liquid medium (9) at 30°C. For germlings, conidia (10^7 cells per ml) were grown (3 h at 30°C) in Vogel's 1× liquid medium. Protoperithecia were prepared by innoculation with a drop of conidial suspension onto a cellophane paper placed on the surface of a Westergaard's plate (9) and growth was allowed to proceed in the light at 25°C until protoperithecia were visible (≈8 days). Cells were scraped from the cellophane and RNA was prepared as described above. All mRNA fractions were purified using Oligotex resin (Qiagen).

Gene Replacement of *nik-1*$^+$ with *his-2*$^+$. pKB49 contains the *his-2*$^+$ gene on a 5-kb *Hin*dIII fragment subcloned from a cosmid provided by Dorsey Stuart (University of Hawaii). The *his-2*$^+$ marker was isolated as a 5-kb *Hin*dIII fragment from pKB49 and the ends were made blunt with Klenow fragment. The fragment of pHK1 from the *Eco*RV site upstream of the *nik-1* translational start site to the *Nhe* I site was removed and the *Nhe* I ends were made blunt with Klenow fragment. The blunt-ended *his-2*$^+$ fragment was ligated into the modified pHK1 to yield pHK1Δ*nik-1::his-2*$^+$. The *Bam*HI fragment of pHK1Δ*nik-1::his-2*$^+$ containing the *his-2*$^+$ insertion was used to electroporate (15) conidia from the Stadler strain (*pdx-1his-2Δmtr*) of *N. crassa* obtained from David Stadler (University of Washington). Transformants were selected for histidine auxotrophy on minimal sorbose plates with regeneration agar (16) containing pyridoxine hydrochloride (pdx) (10 μg/ml). Approximately 50 transformants were picked from plates onto 1× Vogel's minimal medium slants (9) containing pdx (VMpdx). Genomic DNA was isolated from each of these 50 strains (17), digested with *Bgl* II and *Eco*RI, and transferred onto nylon. Blots were probed with a PCR product to the upstream region of *nik-1*$^+$ (nucleotides 325–615). Clones containing the desired bands were then subjected to three or four rounds of plating to isolate homokaryons, which were identified by Southern blot analysis (not shown) as well as PCR of genomic DNA with *nik-1*$^+$-specific primers NK7 (GTCCTCCAAGTACCCTG) and NK16 (GATCAGCTACGGACTTTC). The plasmid pKB49 was electroporated into Stadler and clones were subsequently purified in the same manner to yield clone 49-9-5, which served as the wild-type control. DNA from the mutant and wild-type strains were probed with the *his-2*$^+$ gene to ensure that multiple copies of the gene were not integrated in the genome.

RESULTS

Cloning of *N. crassa nik-1*$^+$. Analysis of the alignment of several members of the histidine kinase family showed that there is a subclass of kinases that contain both a kinase domain and a response regulator domain, termed hybrid kinases (1–3). Two eukaryotic kinases, ETR1 and SLN1, are members of this subclass (4, 6). Degenerate primers corresponding to the H box consensus H(E/D)(M/I/L/F)RTP and N box NLV(S/G)NA(I/V)KFT were designed and used to amplify genomic DNA from *N. crassa*. Two PCR products (*nik-1* and *nik-2*) were obtained that upon sequencing encoded domains homologous to two-component histidine kinases (data not shown). Southern blot analysis of genomic DNA from *N. crassa* with each PCR product showed that each product corresponded to a unique gene (data not shown). We decided to clone the gene corresponding to the *nik-1*$^+$ PCR product and asses its function in *N. crassa*.

The *nik-1*$^+$ PCR product was used as a probe to screen *N. crassa* genomic and cDNA libraries in order to clone the *nik-1*$^+$ gene whose structure is shown in Fig. 1. The 5′ untranslated region is rich in structure and includes an intron of 100 bp (Fig. 1*A*). Also, this region is rich in repetitive nucleotide elements, the first of which is the sequence $(AGTC)_6 \ldots (GATC)_6$, which has the possibility of forming a stem–loop structure. Next, the repeat TACC is found in tandem 10 times followed shortly by two more repeats. This repeat has also been noticed in the 5′ untranslated region of the *nit-3*$^+$ gene in *N. crassa*, although its significance remains unknown (18). The TACC repeat is followed by a purine-rich segment. The four introns found in *nik-1*$^+$ have consensus splice sites that agree with those found in other genes from *N. crassa* (19).

Analysis of the predicted amino acid sequence shows that Nik-1 is a member of the hybrid class of histidine kinases that contain both a kinase and response regulator domain (Fig. 1*B*). The starting AUG is contained within the sequence (GCCCA-

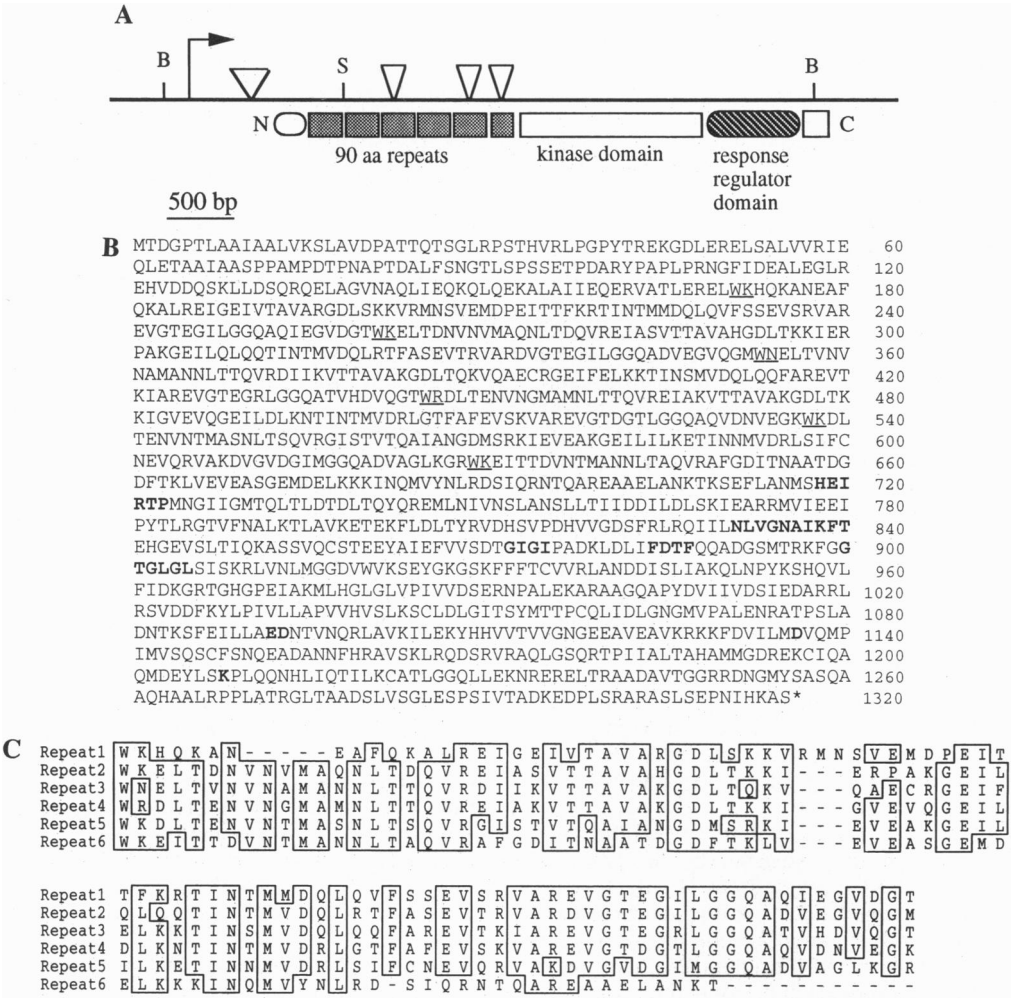

FIG. 1. Gene structure and predicted amino acid sequence of *nik-1*$^+$. (*A*) Schematic representation of the *nik-1*$^+$ coding region in relation to gene structure. Positions of introns are given as inverted triangles above the horizontal line representing the gene. Protein-encoding domains are shown below the line as boxes. Restriction sites are given by single letters: B, *Bam*HI; S, *Sac* I. (*B*) Predicted amino acid sequence of Nik-1. Amino acid residues corresponding to conserved sequences of two-component histidine kinases and response regulators are shown in boldface type. Amino acids at the beginning of each of the six N-terminal repeats is underlined. (*C*) Alignment of each of the 90-amino acid N-terminal repeats of Nik-1.

CAATCATGAC) consistent with other genes in *N. crassa* (20). Nik-1 is most similar to the kinase and regulator regions (57%) of the BarA protein from *Escherichia coli* (21). The function of BarA is not known, but it can act as a multicopy suppressor in a strain that lacks the osmosensor EnvZ (21). The Nik-1 protein is novel in that the N-terminal end of the polypeptide contains a unique 90-amino acid motif, which is repeated 5 times, followed by a short sixth truncated repeat (Fig. 1*C*). The N-terminal repeat region has a high probability of forming a coiled-coil structure when analyzed with the algorithm of Lupas and Stock (22). A computer search using the BLAST program revealed that the N-terminal end of Nik-1 shares homology [P(N), 1.2×10^{-6}] with bacterial sensory transducers, most notably Htr1 from *Halobacterium salinarium* (23). Htr1 couples sensory rhodopsin to a soluble histidine kinase and thus regulates phototaxis. Analysis of the distribution of polar and hydrophobic residues in the amino acid sequence of Nik-1 suggests that the protein is soluble.

Nik-1 Is a Stage-Specific Protein Involved in Hyphal Development. *N. crassa* exhibits both an asexual vegetative and a sexual phase in its life cycle (24). As *N. crassa* grows vegetatively, it forms a branched multicellular network of hyphae called a mycelium. Hyphae are extended from the apical tip and form branches at regular intervals, often fusing with other hyphae. Upon conditions of nutrient deprivation and dessication, the mycelium sends up aerial hyphae, which then differentiate into conidiophores that, in turn, ultimately produce the asexual spores known as conidia, which can also function as male gametes during the sexual cycle (24). Expression of *nik-1*$^+$ was stage specific as the transcript (5.5 kb by Northern

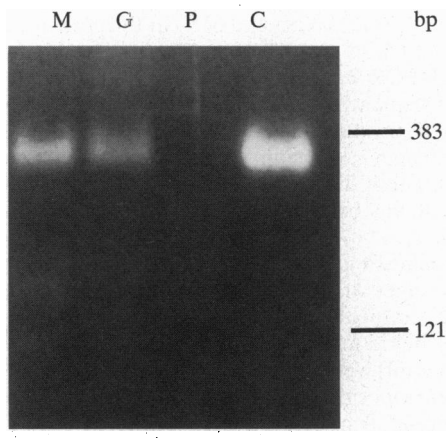

FIG. 2. Agarose gel electrophoresis of *nik-1*-specific RT-PCR products from mRNA at different times during *N. crassa* development. M, mRNA from mycelia at 16 h; G, mRNA from germlings at 3.5 h; P, mRNA from cultures that have differentiated into protoperithecia at 8 days; C, genomic DNA.

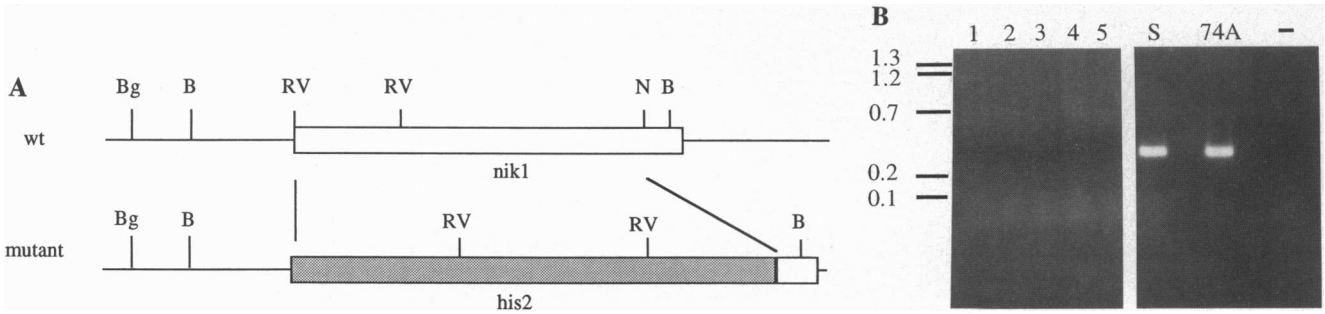

FIG. 3. Construction of the Δ*nik-1* mutant by gene replacement with *his-2*$^+$. (*A*) Schematic representation of the genomic DNA structure of the wild-type and Δ*nik-1* strains. Restriction sites are as follows: Bg, *Bgl* II; B, *Bam*HI; RV, *Eco*RV; N, *Nhe* I. (*B*) Agarose gel electrophoresis of *nik-1*-specific PCR products after amplification of genomic DNA prepared from the Δ*nik-1* mutants. The five independent clones were analyzed after they showed a single band upon Southern hybridization. Lanes: 1, KO-8-1-3; 2, KO-8-1-4; 3, KO14-2-1; 4, KO34-8-6; 5, KO34-10-6; S, *N. crassa* Stadler; 74A, *N. crassa* 74-OR23-1VA; −, control.

analysis; data not shown) could be detected by RT-PCR of mRNA only during the vegetative phase of the *N. crassa* life cycle and not after differentiation into the sexual phase (Fig. 2).

To determine whether Nik-1 is important for fungal development, we constructed a deletion mutant by replacing *nik-1*$^+$ with the *his-2*$^+$ gene (Fig. 3). It was apparent that there were morphological phenotypes associated with the mutant during the vegetative phase. Comparison of the Δ*nik-1* mutant and wild-type hyphal structure is shown in Fig. 4. In the mutant, many of the aerial hyphae become swollen and misshapen and appear to lyse. Macroscopically, aerial hyphae of the mutant have the appearance of cotton candy, grow as a mass that fills the culture tube, and have large visible areas of lysis. Conidia could be formed by the mutant, but they were adherent and not readily dispersed in comparison to wild-type conidia. Finally, upon exposure to light, the mutant turns a deep orange or flame color when grown on small agar slants, whereas the wild type is normally peach colored. Therefore, loss of *nik-1*$^+$ function impairs normal vegetative development in *N. crassa*.

The sexual phase of the growth cycle was examined by placing the mutant on Westergaard's medium in slants. Protoperithecia became visible and these could differentiate into

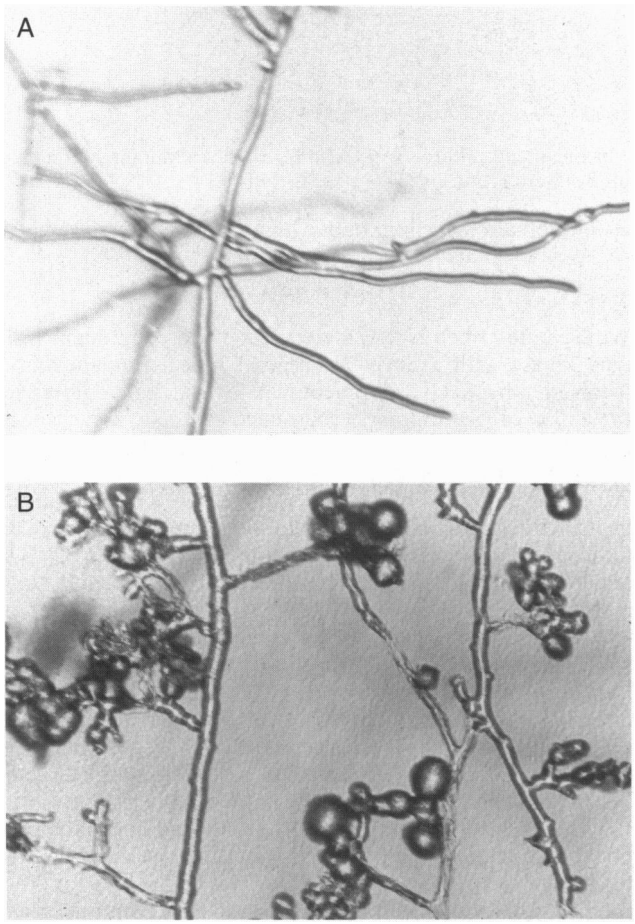

FIG. 4. Comparison of the structure of wild-type hyphae (*A*) and Δ*nik-1* mutant aerial hyphae (*B*) when grown in small agar VMpdx slants (9). Wild-type slant was photographed earlier at 3 days compared to 4.5 days for the mutant so that individual hyphae could be observed in the field. (×96.)

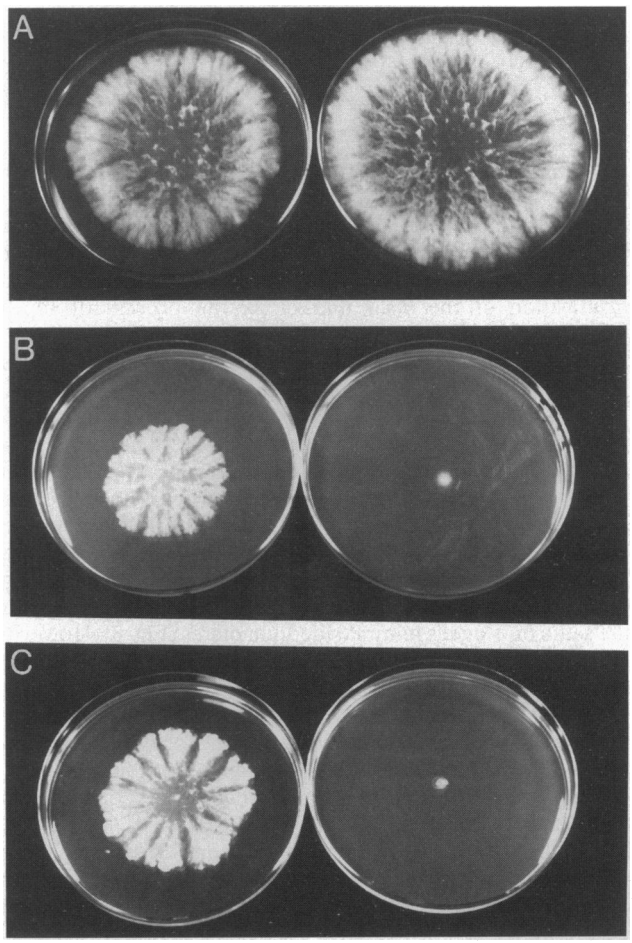

FIG. 5. Comparison of wild-type and Δ*nik-1* mutant grown on VMpdx plates containing no addition (*A*), 0.7 M NaCl (*B*), or 1 M sorbitol (*C*). (*Left*) Wild type. (*Right*) Mutant. Conidia from 5-day-old slants were spotted onto plates and incubated at 30°C for 36 h.

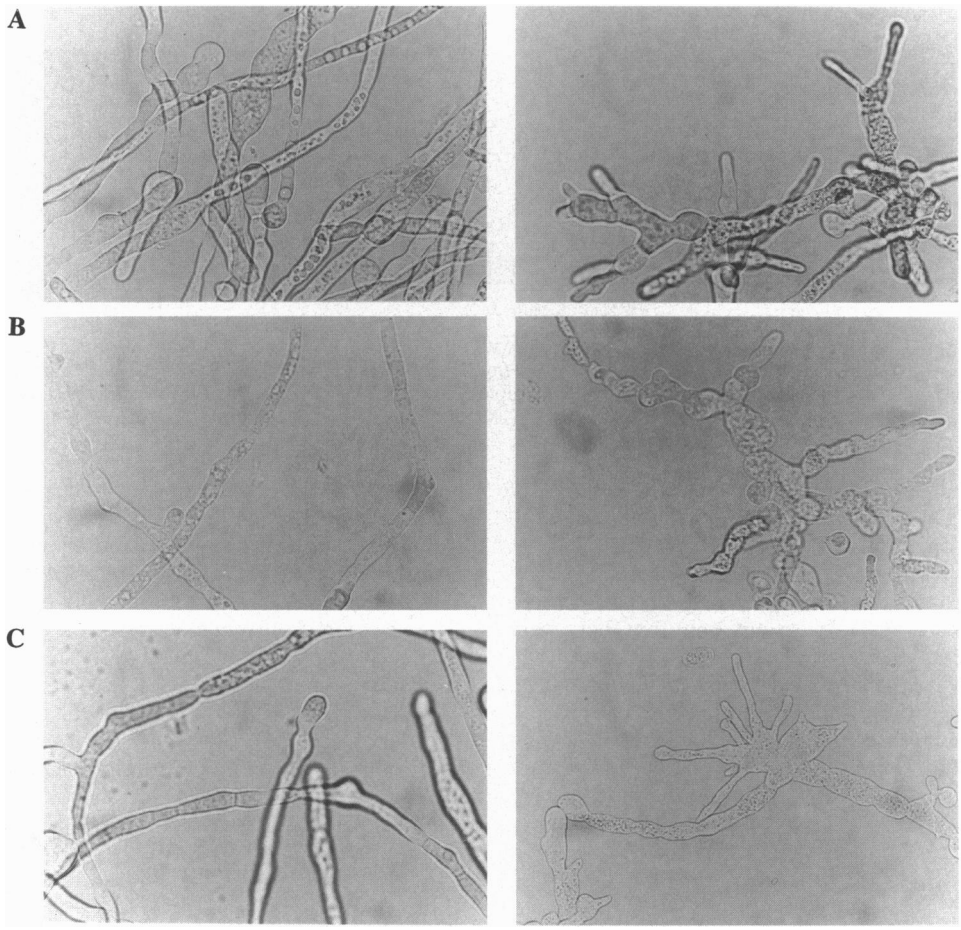

FIG. 6. Comparison of hyphal structure of Δ*nik-1* and wild-type strains in shaking liquid culture. (*Left*) Wild type. (*Right*) Mutant. Medium for growth is VMpdx with 1 M NaCl (*A*), 1 M sorbitol (*B*), or 1 M KCl (*C*). Cultures were grown for 24 h at 30°C. (×800.)

perithecia upon fertilization with conidia from a wild-type strain, 74A (data not shown). Spores could be obtained from this cross but at reduced numbers, which we think is due to inactivation of the *his-2* marker by the process of repeat induced point mutation in *N. crassa* (25). The ability of Δ*nik-1* mutants to be fertilized is consistent with the expression pattern of the gene during development.

Δ*nik-1* Mutants Are Osmosensitive. The characteristics of the Δ*nik-1* mutant resemble those of osmosensitive *N. crassa* mutants whose morphologies are drastically affected by humidity (26). We have observed that the morphology of the Δ*nik-1* mutant appears closer to that of wild type when cultures are grown in large flasks or slants as opposed to small slants. Therefore, we tested the tolerance of the Δ*nik-1* mutant to different osmolytes. Growth of the mutant and wild type was the same on Vogel's minimal medium plates (Fig. 5). However, growth on 1 M sorbitol/0.7 M NaCl, (or 1 M KCl; data not shown) resulted in restricted colonial growth, hyphae were excessively branched and bumpy, and aerial hyphae were nonexistent, resulting in a subsequent abrogation of conidia formation (Fig. 5). Therefore, it was apparent that the *nik-1*$^+$ deletion manifested itself dramatically under conditions of high osmostress.

We also monitored the growth response of the mutant in shaking liquid culture. Normally *N. crassa* can grow as a mycelium in submerged shaking liquid culture although it does not conidiate (24). Wild type grows as a mycelium with the addition of 1 M sorbitol, NaCl, or KCl. In contrast, growth of the mutant was significantly impaired and it tended to form small clumps of irregular-shaped hyphae that were hyperbranched and swollen (Fig. 6). Hence, the mutant did not form a well-defined mycelium under high osmotic stress.

DISCUSSION

We have described Nik-1, a eukaryotic two-component histidine kinase with a novel N-terminal repeat domain that is involved in hyphal development in *N. crassa*. It is required for the ability of *Neurospora* to reproduce asexually under conditions of high osmostress, which in its natural environment may be important for colonization of new locations by the fungus. We believe that involvement of proteins like Nik-1 in proper hyphal development is likely to be a common theme in other filamentous fungi, as we have detected a *nik-1*$^+$ homolog (89% amino acid identity in the kinase domain) in a related Ascomycete, *Aspergillus nidulans*. So far, eukaryotic members of the two-component family have been found only in organisms with a cell wall (plants and fungi; this work) (4, 5, 6, 7, 8). Furthermore, we have used multiple H and N box primers and have not found homologous proteins in mammalian systems. Therefore, components of these signaling pathways may provide an excellent target for development of antibiotics directed against both fungal and bacterial pathogens in mammals.

The fact that deletion of *nik-1*$^+$ results is a morphological phenotype suggests that there may be a defect in cell wall or membrane integrity as has been shown to be the case for other morphological mutants in *Neurospora* (27, 28). This could lead to an osmosensitive phenotype as a secondary effect. We have evidence that this may be the case as the Δ*nik-1* mutant grown in the presence of high salt does not stain with Calcofluor, a dye known to bind to the chitin component of cell walls (L.A.A. and M.I.S., unpublished results; ref. 29). On the other hand,

Nik-1 may be an osmosensor in *N. crassa*. The process of building a filamentous network and subsequent aerial hyphal network involves complex regulation of many genes and their products. Experiments are now in progress to define the other components of the signal transduction pathway involving Nik-1.

We would like to thank D. Stuart for the *his-2*$^+$ cosmid, D. Stadler for the *N. crassa* strain Stadler, and G. Turner for the gift of *N. crassa* genomic DNA from 74A. We would also like to thank L. Brundage and G. Sanna for reading the manuscript and R. Metzenberg for helpful suggestions. This work was supported by a grant from the National Institutes of Health (AI 19296).

1. Alex, L. A. & Simon, M. I. (1994) *Trends Genet.* **10,** 133–138.
2. Swanson, R. V., Alex, L. A. & Simon, M. I. (1994) *Trends Biochem. Sci.* **19,** 485–490.
3. Parkinson, J. S. & Kofoid, E. C. (1992) *Annu. Rev. Genet.* **26,** 71–112.
4. Chang, C., Kwok, S. F., Bleecker, A. B. & Meyerowitz, E. M. (1993) *Science* **262,** 539–544.
5. Hua, J., Chang, C., Sun, Q. & Meyerowitz, E. M. (1995) *Science* **269,** 1712–1714.
6. Ota, I. M. & Varshavsky, A. (1993) *Science* **262,** 566–569.
7. Maeda, T., Wurgler-Murphy, S. M. & Saito, H. (1994) *Nature (London)* **369,** 242–245.
8. Brown, J. L., North, S. & Bussey, H. (1993) *J. Bacteriol.* **175,** 6908–6915.
9. Davis, R. H. & deSerres, F. J. (1970) *Methods Enzymol.* **17,** 79–143.
10. Ausubel, F. M., ed. (1994) *Current Protocols in Molecular Biology* (Wiley, New York).
11. Aatsinki, J. T., Lakkakorpi, J. T., Pietila, E. M. & Rajaniemi, H. J. (1994) *BioTechniques* **16,** 282–288.
12. Dale, R. M., McClure, B. A. & Houchins, J. P. (1985) *Plasmid* **13,** 31–41.
13. Orbach, M. J., Sachs, M. S. & Yanofsky, C. (1990) *J. Biol. Chem.* **265,** 10981–10987.
14. Reinert, W. R., Patel, V. B. & Giles, N. H. (1981) *Mol. Cell. Biol.* **1,** 829–835.
15. Vann, D. C. (1995) *Neurospora Newslett.* **42,** 53.
16. Case, M. E., Schweizer, M., Kushner, S. R. & Giles, N. H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5259–5263.
17. Metzenberg, R. L. & Stevens, J. N. (1982) *Neurospora Newslett.* **35,** 28.
18. Okamoto, P. M., Fu, Y. H. & Marzluf, G. A. (1991) *Mol. Gen. Genet.* **227,** 213–223.
19. Bruchez, J. J. P., Eberle, J. & Russo, V. E. A. (1993) *Fungal Genet. Newslett.* **40,** 89–96.
20. Bruchez, J. J. P., Eberle, J. & Russo, V. E. A. (1993) *Fungal Genet. Newslett.* **40,** 85–88.
21. Nagasawa, S., Tokishita, S., Aiba, H. & Mizuno, T. (1992) *Mol. Microbiol.* **6,** 799–807.
22. Lupas, A., Van Dyke, M. & Stock, J. B. (1992) *Science* **252,** 1162–1164.
23. Yao, V. J. & Spudich, J. L. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11915–11919.
24. Springer, M. L. (1993) *BioEssays* **15,** 365–374.
25. Selker, E. U. (1990) *Annu. Rev. Genet.* **24,** 579–613.
26. Perkins, D. D., Radford, A., Newmeyer, D. & Björkman, M. (1982) *Microbiol. Rev.* **46,** 426–570.
27. Perkins, D. D. & Barry, E. G. (1977) *Adv. Genet.* **19,** 133–285.
28. Mishra, N. C. (1977) *Adv. Genet.* **19,** 341–405.
29. Maeda, H. & Ishida, L. (1967) *J. Biochem. (Tokyo)* **62,** 276–278.